

SOLUBILITY OF CARNOSIC ACID IN SUPERCRITICAL CO₂ + ETHANOL AS A MODIFIER: MEASUREMENTS AND THERMODYNAMIC MODELLING

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Antioxidants are widely used in food products to preserve deterioration of their lipid components. Lately the nutraceutical properties of such compounds have driven much attention for their relationship with human health, and then natural antioxidants are preferred. Among natural antioxidants, rosemary (*rosmarinus officinalis* L.) has been described as one of the spices with higher antioxidant activity, which has been mainly attributed to carnosic acid content [1].

The present study is devoted to the experimental measurement and thermodynamic modeling of solid carnosic acid solubility in supercritical CO₂ + ethanol as a co-solvent. Measurements were carried out at temperatures in the range of 313.15 K and 333.15 K, pressures ranging from 280 to 400 bar, and at different content of the modifier ethanol (from 0.7% to 10%).

The Group Contribution Associating Equation of State (GCA-EoS) [2] was applied in this work to represent the experimental solubility data obtained.

INTRODUCTION

Rosmarinus officinalis L. and *Salvia officinalis* L. have been shown to have the strongest antioxidant activities among herbs [3]. Some of their phenolic compounds have active oxygens that can inhibit lipid peroxidation and therefore the corresponding extracts have been widely used to stabilize fat and fat-containing foods [4]. Several authors have demonstrated the high antioxidant activity of some rosemary's phenolic acids, phenolic diterpenes and flavonoids, namely, rosmarinic acid, carnosic acid, and carnosol. However, it has also been demonstrated that some of the most effective antioxidant compounds, like carnosic acid and carnosol, are highly unstable depending of factors like temperature, light, oxygen, and solvent used in the extraction [5-7].

Supercritical Fluid Extraction (SFE) has important advantages over conventional extraction techniques. The absence of both light and air during the extraction process along with the low operating temperatures can reduce the incidence of degradation reactions, while the use of CO₂ as extracting agent can allow the work in an environmentally clean media [8].

Pure carnosic acid can be isolated from rosemary extracts using SFE followed by a purifying step with, for example, Supercritical Fluid Chromatography [9]. The key factor in this separation technique is the possibility of adjusting the density-based solvent power of the supercritical mobile phase. Then, knowledge of carnosic acid solubility in the supercritical phase is important in order to analyze and optimize the separation process.

I – EXPERIMENTAL

Chemicals.

Carnosic acid (97 mass %, GC grade) was purchased from Aldrich Chemical Co. Inc. Absolute ethanol (99.8 mass %, GC grade), supplied by Prolabo S.A. was used as a modifier and solvent to collect the extract. The reagents were used without further purification since chromatography did not show any significant impurities. High-purity CO₂ (more than 99.9 vol % purity, SFC grade) supplied by AIR LIQUIDE was used as received. Silica extra pure (more than 99.8 mass %) from Sigma Chemical Co. Inc. was utilized as support of products.

Equipment and procedure.

The solubilities were measured with an SFX 3560 extractor with two syringe pumps (Model 260D) manufactured by ISCO (Lincoln, NE, USA). The extractor measured the amount of supercritical CO₂ and ethanol modifier used during the extraction. The flow rate of the supercritical fluid used for all experiments was 0.5 mL/min, which ensured that saturation of the supercritical phase was achieved in a reasonable time. Ethanol was used to trap the carnosic acid extracted. A 8453 Hewlett-Packard diode array UV-Vis spectrophotometer was used to determine the amount of carnosic acid in the extract, after calibration with gravimetrically prepared standard solutions.

Experimental results.

Rosemary antioxidants and phenolic compounds are barely removed using pure CO₂ in the SFE process. The use of small quantities of an organic solvent as a modifier can significantly improve phenols extraction [2]. In this work, ethanol was used as a modifier to increase carnosic acid solubility in the supercritical phase. Ethanol was chosen because is a polar solvent, its use is allowed in the food industry, and it could be easily removed from the extract by evaporation at relatively low temperatures.

The solubilities of carnosic acid in the supercritical phase were measured at 313, 323 and 333 K, pressures ranging from 280 to 400 bar, and for different CO₂ + ethanol mixtures, with the ethanol content ranging from 0.7% to 10%. The latter was done in order to explore the optimization of carnosic acid extraction with a minimum consumption of co-solvent. The results obtained are shown in Figures 1 and 2.

Figure 1: Experimental carnosic acid solubilities with 6% ethanol modifier: (●) 313 K, (Δ) 323 K and (■) 333 K. Dotted line: GCA-EoS optimal fit

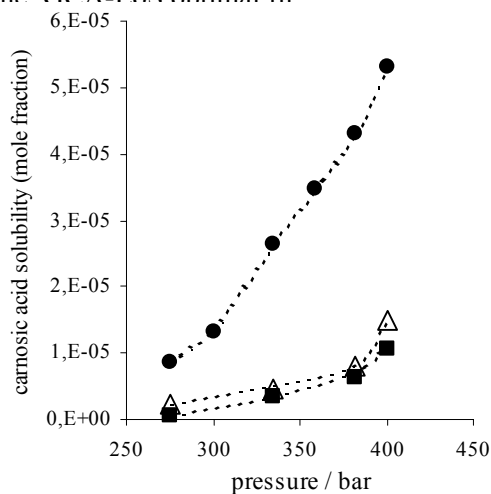
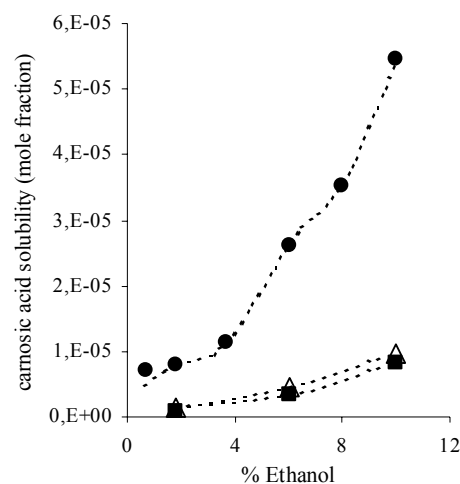


Figure 2: Experimental carnosic acid solubilities at 335 bar: (●) 313 K, (Δ) 323 K and (■) 333 K. Dotted line: GCA-EoS optimal fit.



According to the experiments the solubility of carnosic acid increases with pressure (Fig. 1) and with the amount of ethanol (Fig. 2). Also, in the range of pressures explored, solubilities are higher at lower temperatures; the solvating power of CO₂ becomes greater and more solute is transferred to the supercritical phase.

II – THERMODYNAMIC MODELLING

The solubility of the supercritical fluid (CO₂ + Ethanol) in the solid phase can be considered negligible. Thus, the carnosic acid mole fraction (y_s) in the supercritical phase (SCP) is given by:

$$y_s = \frac{P_s^{\text{sat}}}{P} E \quad (1)$$

where P_s^{sat} is the sublimation (vapour) pressure of the pure solid, P is the pressure, the ratio P_s^{sat} / P is the ideal solubility and E is the enhancement factor, given by:

$$E = \frac{\varphi_s^{\text{sat}} \exp\left[\frac{v_s^{\text{solid}}(P - P_s^{\text{sat}})}{RT}\right]}{\varphi_s^{\text{SCP}}} \quad (2)$$

The exponential term in Eq. (2) is the Poynting correction, assuming a constant value for the solid molar volume (v_s^{solid}). This term gives the effect of pressure on the pure solid fugacity, but it generally accounts for an enhancement factor less than 2 or 3. Since P_s^{sat} is quite small, the carnosic acid fugacity coefficient evaluated at the sublimation pressure is nearly equal to the unity ($\varphi_s^{\text{sat}} \approx 1$). However, the solute fugacity coefficient in the supercritical phase (φ_s^{SCP}) is always far removed from unity and can produce very large enhancement factors. Thus, the pure solid P_s^{sat} values and the φ_s^{SCP} calculations, using a thermodynamic model, are the key factors in the thermodynamic modelling of solid carnosic acid solubilities in the SC-CO₂ + Ethanol phase.

Due to the lack of information about carnosic acid sublimation pressures found in the literature, correlative and/or predictive methods have to be used in order to estimate the corresponding P_s^{sat} values. On the other side, φ_s^{SCP} values were calculated in this work using the GCA-EoS [2], an upgraded version of the GC-EoS developed by Skjold-Jørgensen [10].

Carnosic acid physical properties.

To calculate the solubility of carnosic acid in the SCF mixture using Eqs. (1) and (2) and the GCA-EoS model, the following pure component physical properties must be known: sublimation pressure, solid volume and critical properties. However, these parameters could not be found in the literature, or are experimentally unattainable, and have to be estimated.

Applying Fedors group contribution method [11] the carnosic acid critical temperature was estimated to be 950.5 K. The sublimation pressure and solid volume of carnosic acid were estimated by correlating experimental data [12-13] for the solid volumes and sublimation pressures of high-molecular weight *n*-alkane, *n*-alcohol, phenolic and aromatic compounds. The carnosic acid parameters resulted from these correlations were used in the solubility calculations and are reported in Table 1.

The Group Contribution Association Equation of State (GCA-EoS).

In the GCA-EoS model, the residual Helmholtz energy (A^{res}) is described by three terms, a free volume, a contribution from dispersive attractive intermolecular forces and a contribution from group association effects:

$$A^{res} = A^{fv} + A^{att} + A^{assoc} \quad (3)$$

The free volume contribution A^{fv} is modelled assuming hard-sphere behaviour for the molecules (a Carnahan-Starling type term), characterizing each substance i by a hard-sphere temperature dependent diameter d_i :

$$d_i = 1.065655 d_{ci} \{1 - 0.12 \exp[-2T_{ci}/(3T)]\} \quad (4)$$

where d_{ci} is the value of the hard-sphere diameter at the critical point of pure component i , and is given by:

$$d_{ci} = (0.08943RT_{ci} / P_{ci})^{1/3} \quad (5)$$

Table 1: Carnosic Acid physical properties used in this work.

physical property	
critical temperature, T_c	950.5 K
solid volume, v_s^{solid}	393.03 cm ³ /mol
sublimation pressure, P_s^{sat}	8.22 × 10 ⁻¹¹ bar at 313 K
	5.76 × 10 ⁻¹⁰ bar at 323 K
	9.34 × 10 ⁻⁰⁹ bar at 333 K

The attractive contribution to the Helmholtz energy is based on the NRTL model and accounts for dispersive attractive forces through binary interaction parameters between the constituent molecular groups. The A^{assoc} contribution takes into account association effects through a group contribution approach based on Wertheim Statistical Association Fluid Theory. A detailed description of the GCA-EoS model can be found elsewhere [2].

GCA-EoS parameters and modelling results.

The carnosic acid molecular structure is showed in Figure 3. The constituent functional groups present in the carnosic acid + CO₂ + Ethanol mixture are: phenol ACOH, carboxylic acid COOH, alcohol CH₂OH, CO₂, plus parafinic (CH₃, CYCH₂, CYCH) and aromatic (ACH, AC) groups. Pure group parameters and binary attractive and associative parameters have been reported in the literature [2, 10, 14-16], except for the carboxylic acid - phenol and the CO₂ - phenol attractive interactions.

The hard sphere diameter in the GCA-EoS model is considered to be temperature dependent (see Eq. 4), and the d_c value is generally calculated from pure compound critical properties or fitting vapour pressure data. Due to the uncertainties in the carnosic acid critical parameters and its very low volatility, its d_c value was considered as a fitting parameter.

Simultaneously fitting of the carboxylic acid - phenol and the CO₂ - phenol attractive interaction parameters and the carnosic acid d_c value, in order to minimize the following standard objective function:

$$F = \sum_{i=1}^{N_{exp}} \left(\frac{y_s^{exp} - y_s^{cal}}{y_s^{exp}} \right)^2 \quad (6)$$

could not provide a quantitative representation of the solubilities measured experimentally. Further optimisation procedures, including new sets of binary interaction parameters but using an unique solute d_c value, could not improve the thermodynamic modelling solubility

calculations. Also, calculations were not satisfactory when considering the solute sublimation pressure as an optimisation variable.

Figure 3: Carnosic acid molecular structure.

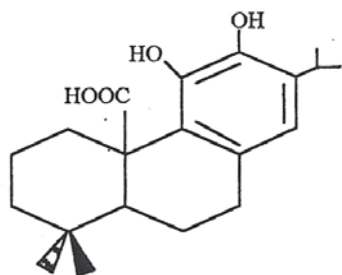


Figure 4: Optimal carnosic acid hard-sphere diameter d calculated for each experimental data point: (●) 313 K, (Δ) 323 K and (■) 333 K. Z^{SCP} is the SC phase compressibility factor. Solid line: lineal regression ($R^2=0.95$).

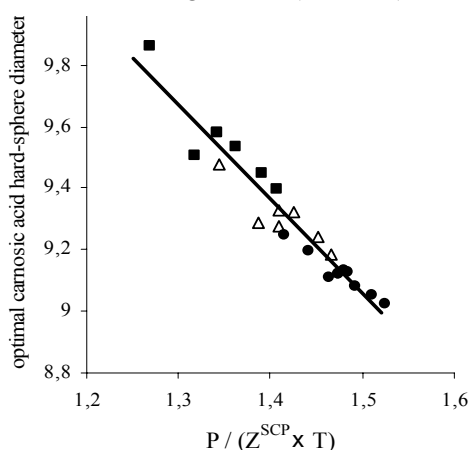


Figure 5: Exp. carnosic acid solubilities with 6% ethanol modifier: (●) 313 K, (Δ) 323 K and (■) 333 K. Solid lines: GCA-EoS calculations with a temperature and pressure dependent solute hard-sphere diameter.

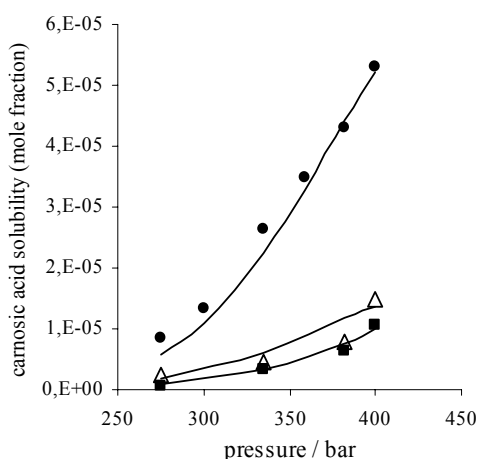
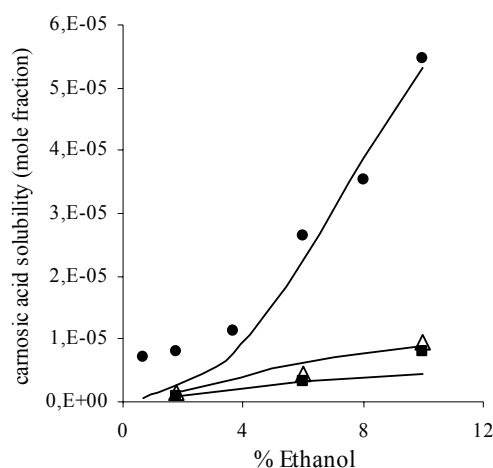


Figure 6: Exp. carnosic acid solubilities at 335 bar: (●) 313 K, (Δ) 323 K and (■) 333 K. Solid lines: GCA-EoS calculations with a temperature and pressure dependent solute hard-sphere diameter.



Based on previous works [17-18] the optimal carnosic acid hard-sphere diameter (d) for *each* experimental data point was calculated. These values, which obviously provide a satisfactory correlation of the experimental data (dotted lines in Fig. 1 and 2), showed to be dependent on the supercritical phase density as can be observed in Figure 4. These optimal carnosic acid d values obtained were correlated with the reduced temperature and pressure. Using this correlation the solubility calculations were considerably improved in order to

represent the experimental observed solubility variation with both pressure (Fig. 5) and ethanol content (Fig. 6).

CONCLUSIONS

In the present study the solubility of carnosic acid in SC-CO₂, with ethanol as a modifier, is reported. The experimental data measured shows the variation of carnosic acid solubility with temperature, pressure and ethanol content, in a wide range of SFE operation conditions. The GCA-EoS was used to correlate the experimental data. Due to the lack of information in the literature about the parameters of pure carnosic acid, an estimation was performed. The key step in the modelling proved to be the introduction of a solute hard-sphere parameter in the Carnahan-Starling repulsive term of the model, which depends not only on temperature (Eq. 4) but also on pressure.

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